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Research Note

Application of Carnatrol™ and Timsen™ to Decontaminate Beef†

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ABSTRACT

The spray application of two commercial decontaminating agents for reducing bacterial populations associated with fecal contamination on beef was examined in two separate experiments. Individual pieces of prerigor lean beef tissue were inoculated with fresh bovine feces and subjected to a 15-s spray wash (75 lb/in^2, 20°C) with water or various concentrations of Carnatrol™, composed of copper sulfate pentahydrate, or Timsen™, 40% *N*-alkyldimethylbenzylammonium chloride in 60% stabilized urea, and stored under refrigerated (5°C) conditions. When Carnatrol™ was applied to beef tissue at 20, 40, and 80 ppm, bacterial populations were not statistically different ($P \geq 0.05$) than water-treated populations at days 0, 1, and 2. When Carnatrol™ was applied to tissues at 160 ppm, bacterial populations were statistically different ($P \leq 0.05$) from water-treated tissue on all of the days examined; however, reductions were not greater than 0.58, 0.42, and 0.35 log CFU/cm^2 at days 0, 1, and 2, respectively. Remaining bacterial populations resulting from spray applications of Timsen™ to tissues at 200, 400, and 800 ppm were not statistically different than remaining bacterial populations of water-treated tissues at days 0, 1, 2, or 3. Reductions in bacterial populations associated with Timsen™ were no greater than 0.40 log CFU/cm^2 on any of the days examined. This study demonstrates that under conditions used in this study, spray washes with either of the two commercially available decontaminating agents were no more effective than water washes for reducing bacterial populations associated with fecal contamination on beef tissue.

Key words: Beef, copper sulfate, quaternary ammonium, decontamination

Beef carcasses may be decontaminated with antimicrobials as a means of reducing the load of pathogenic and spoilage bacteria; for a review, see *(4)*. While residual activity of many antimicrobials has been reported, most decontamination studies only have investigated the effects of these compounds on bacterial populations immediately after treatment *(1, 2, 3, 5)*. Two commercial compounds, Carnatrol™ and Timsen™, are known to exhibit immediate and residual activity when applied to water or food-contact surfaces, respectively (manufacturers' information). The active ingredient of Carnatrol™ is copper sulfate pentahydrate (Bactrol Laboratories, Ft. Myers, FL) and the active ingredient of Timsen™ is 40% *N*-alkyldimethylbenzylammonium chloride in 60% stabilized urea (United Promotions, Inc., Atlanta, GA). The purpose of this study was to determine if spray treatments with these two decontaminants elicit antimicrobial activity against bacteria associated with fecal contamination on beef surfaces. The experiments were developed to determine the effects against bacterial populations immediately after spray application as well as after refrigerated storage for up to 3 days.

MATERIALS AND METHODS

Inoculation of beef with bovine feces

Lean beef carcass tissue (BCT; cutaneous trunci) was removed from the outer surface of prerigor (15 min postexsanguination) beef carcasses, trimmed to 10 by 10 by 0.5 cm pieces and surface-sterilized by ultraviolet light *(2)*. Fresh bovine feces were obtained from 12 individual cows fed a corn-silage ration and diluted 1:2 in sterile distilled water. Individual pieces of surface-sterilized beef tissue were completely inoculated with diluted feces by painting the suspension onto the tissue with a 2-in. (5.08-cm) sterile paintbrush and incubating for 15 min at 25°C *(6)*. Populations of approximately 5.50 log CFU/cm^2 were obtained using this methodology.

Spray washing

The model carcass washer (MCW) located at the Roman L. Hruska U.S. Meat Animal Research Center was used to apply distilled water and the antimicrobial agents to the surfaces of the fecally inoculated BCT *(2, 3)*. As directed by the manufacturers, Carnatrol™ (Bactrol Laboratories, Fort Myers, FL) was diluted in distilled water to achieve 20, 40, 80, and 160 ppm active compound, while Timsen™ (United Promotions, Inc., Atlanta, GA) was mixed in distilled water to achieve 200, 400, and 800 ppm

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† Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

active compound for spraying in the MCW. Individual pieces of feces-inoculated lean BCT were subjected to spray treatments with water or with the different concentrations of Carnatrol™ or Timsen™ as follows: pressure, 75 lb/in^2; flow rate, 4.8 liters/min; time, 15 s; temperature, 20°C; oscillation, 60 cycles/min; tissue 8 in. from spray nozzle (Spraying Systems, Co., Wheaton, IL), set at 30° angle. BCT inoculated with feces but not subjected to spray treatments (untreated samples) was handled similarly. For experiments with Carnatrol™ a 25-cm^2 piece was aseptically excised from each piece of BCT, placed into a Sterefil Stomacher bag (Spiral Biotech, Bethesda, MD), and stored at 4°C for 24 or 48 h. For experiments with Timsen™, BCT that was left untreated or subjected to spray treatments was placed on a sterile tray, loosely covered with sterile plastic, and stored at 4°C for 24, 48, or 72 h. On each sampling day, a 25-cm^2 piece was excised and placed into a Stomacher bag. Day 0 samples from either experiment were prepared for bacterial enumeration within 1.5 h after spray treatment. After excision of the 25-cm^2 section, remaining pieces of untreated and treated BCT were used to assess surface pH values (flat electrode, Corning Instruments, Corning, NY).

Bacterial enumeration

Each 25-cm^2 piece of BCT was pummeled for 2 min in 25 ml of buffered peptone water (BPW, pH 7.0) (Difco Laboratories, Detroit, MI) with 0.1% Tween 20. Dilutions of stomachates were made in BPW and samples were plated in duplicate on Trypticase soy agar (TSA) (BBL, Cockeysville, MD) using a Model D Spiral Plater (Spiral Biotech, Bethesda, MD). Plates were enumerated with the CASBA IV Image Analyzer (Spiral Biotech) after incubation for 36 h at 35°C.

Statistical analyses

The Carnatrol™ experiment was a 6 (treatments) by 3 (days) by 6 (replications) factorially arranged, completely randomized design. The Timsen™ experiment was a 5 (treatments) by 4 (days) by 6 (replications) factorially arranged, completely randomized design. Bacterial populations were converted from CFU/ml to CFU/cm^2 prior to statistical analyses. Analysis of variance (ANOVA) was performed on population and surface pH data using the general linear model (GLM) procedure of SAS (SAS Institute, ver. 6.06.01, 1989, SAS Inst., Inc., Cary, NC, 1982) or Instat™ GraphPad (San Diego, CA). The probability level for population or surface pH data was $P \leq 0.05$, unless otherwise noted.

RESULTS AND DISCUSSION

Spray treatments with 20, 40, and 80 ppm Carnatrol™ resulted in microbiological populations statistically similar to results after water sprays at days 0, 1, or 2 (Figure 1). Only spray treatments with 160 ppm resulted in significantly different populations at any of the days examined; however, log-unit reductions were no greater than 0.35 by day 2.

The pH values of solutions and Carnatrol™-treated beef tissue indicated that as the concentration of Carnatrol™ increased, the pH decreased (Table 1). Throughout the experiment, surface pH values of samples treated with 160 ppm were statistically lower than untreated or water-treated tissues, indicating that there was a sustained pH effect attributable to the compound. As suggested by the initial pH of the solutions, it is possible that some of the antimicrobial effect of Carnatrol™ may be due to its acidic properties. A similar study in our laboratories demonstrated that bacterial reductions were correlated to reduced surface pH following application of organic acids and 24-h refrigerated storage ($r = 0.86$) *(2)*.

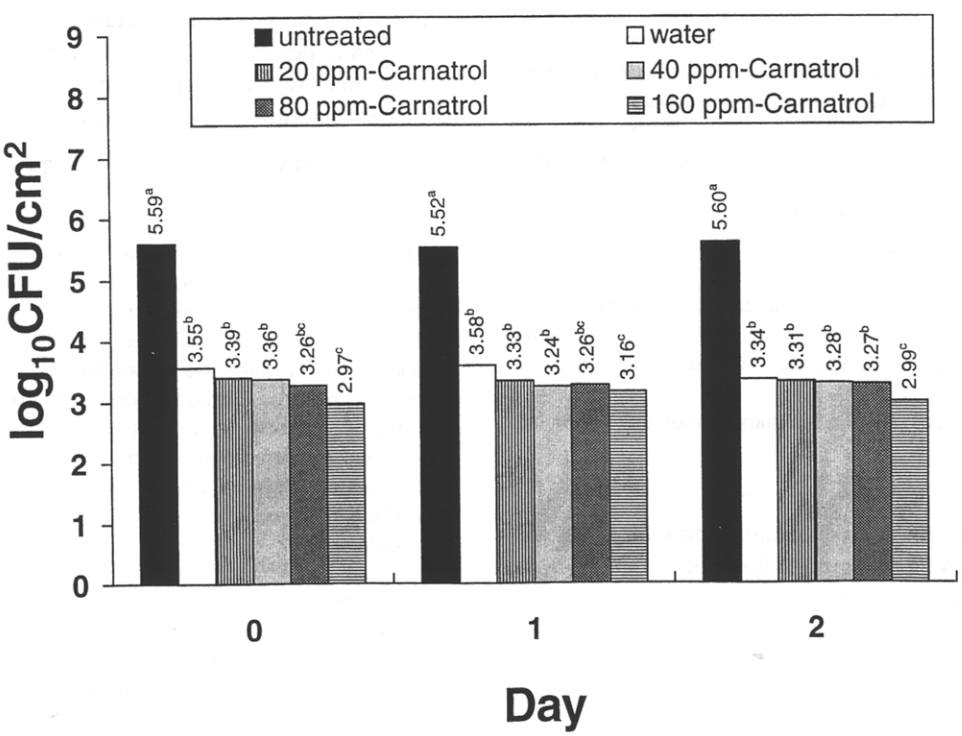

FIGURE 1. *Application of various concentrations of Carnatrol™ to reduce bacterial populations associated with fecal contamination on beef after 0, 1, and 2 days of refrigerated storage. Different letters within day denote statistical significance between treatments* ($P \leq 0.05$). *Each value is the mean of 6 replications.*

TABLE 1. *pH values of solutions and beef following spray treatments with water or Carnatrol™ and refrigerated storage*

Carnatrol™ treatment (ppm)	pH on day: Solution only	Beef carcass tissue		
	0	0	1	2
None	NA[a]	6.94A[b]	6.54A	6.21A
None (d H_2O)	7.20	6.81A	6.09B	5.99B
20	2.84	5.94B	6.19B	5.86B
40	2.54	5.30CD	5.83C	5.82BC
80	2.27	5.33C	5.75CD	5.79C
160	2.03	4.31D	5.58D	5.66C

[a] NA, not applicable.

[b] Different letters within a column denote statistical differences between treatments ($P \leq 0.05$). Each value is the mean of 6 replications.

TABLE 2. *pH values of solutions and beef following spray treatments with water or Timsen™ and refrigerated storage*

Timsen™ treatment (ppm)	pH on day: Solution only	Beef carcass tissue			
	0	0	1	2	3
None	NA[a]	7.10A[b]	6.76A	6.57A	6.65A
None (d H_2O)	7.14	6.63B	6.43A	6.28A	6.24A
200	7.00	6.84B	6.55A	6.64A	6.37A
400	6.95	6.76B	6.56A	6.26A	6.41A
800	6.85	6.91B	6.58A	6.62A	6.49A

[a] NA, Not applicable.

[b] Different letters within a column denote statistical differences between treatments ($P \leq 0.05$). Each value is the mean of 6 replications.

Bacterial populations on beef tissue following spray treatments with any of the concentrations of Timsen™ were not statistically different from water-treated populations on any of the days examined (Figure 2). Unlike Carnatrol™, the pH values of Timsen™ solutions were at or near neutrality (Table 2). While applications of Timsen™ did significantly alter the surface pH of the beef tissue at day 0, the surface pH values were not statistically different than pH values following water washes. Surface pH values of water-treated and Timsen™-treated beef were not statistically different from each other or from untreated tissues after 1, 2, or 3 days of refrigerated storage. Slight reductions with water or Timsen™ may be attributed to the physical removal of bacteria during the spraying process.

Given the minimal reductions in bacterial numbers observed in this study, it is possible that the active compound(s) in Carnatrol™ or Timsen™ were bound to organic material found on the beef carcass surface. Binding of active compounds to organic material may occur when chlorinated compounds are applied to surfaces and/or water with a high organic load (blood, feces, hair, etc.). In such cases, undesirable compounds such as chloramines form which interfere with the activity of the chlorine against bacterial populations *(3, 7)*. As has been observed with chlorinated compounds, the application of higher concentrations of Carnatrol™ or Timsen™ may result in formation of undesirable compounds and/or reduced activity.

Presently, commercial slaughterhouses rinse meat-animal carcasses with antimicrobials after trimming to reduce bacterial contamination. However, in this study, we subjected fecally contaminated beef surfaces to spray washes with Carnatrol™ and Timsen™ to represent a worst-case scenario in which untrimmed areas were subjected to the

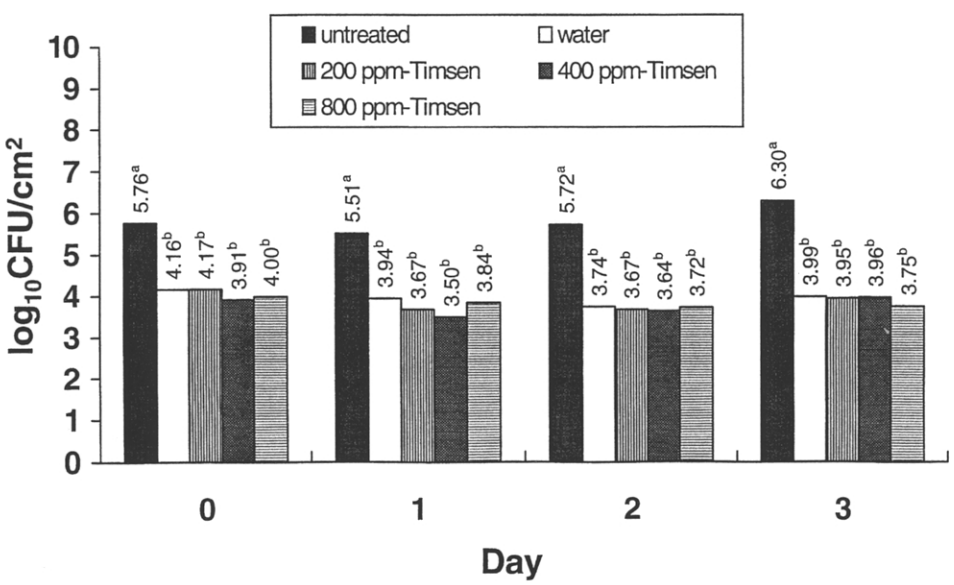

FIGURE 2. *Application of various concentrations of Timsen™ to reduce bacterial populations associated with fecal contamination on beef after 0, 1, 2, and 3 days of refrigerated storage. Different letters within day denote statistical significance between treatments ($P \leq 0.05$). Each value is the mean of 6 replications.*

compounds. While Carnatrol™ and Timsen™ may be effective decontaminants of water or food-contact surfaces (manufacturers' information), our study demonstrates that spray washing with either compound at the specified concentrations did not substantially reduce bacterial populations resulting from fecal contamination on beef.

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